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The methanol and acetone cell extracts displayed similar cytotoxic and ichthyotoxic activity. HPLC analysis of these extracts confirmed that the same compounds were present in both solvent fractions. Subsequent cell mass toxin extraction methodology was carried out with only acetone extraction (3×) followed by syringe filtration. This acetone extract was further purified using repeated acetone extraction and mass fractionated HPLC/MS.

The isolated toxin is a relatively non-polar compound exhibiting maximal absorbance at 238 nm in the UV spectral region. Purification was difficult as several stereo and molecular isomers were present. Active fractions exhibited a strong mass fingerprint at 288 amu which was subsequently shown to be the molecular ion minus an OH functionality (lost as H₂O). MS analyses provided a mass fingerprint common to all of the bioactive fractions (288 [MH-H₂O]⁺; 306 MH⁺) confirming the isomeric nature of the toxic substances. The toxin exists as a 2,6-disubstituted piperidine ring (FIG. 1). The major isomer was shown to have a cis-configuration with respect to C2 and C6, with minor components including trans configuration. COSY experiments on NMR revealed the presence of four stereoisomers under two chiral centers. At this time, only relative stereochemistry is known (Table 1).

TABLE 1

NMR results from euglenophycin analyses.					
Position	¹³ C	APT Multiplicity	Selected H	J	NOESY
1					
2	67.3	CH			
3	22	CH ₂	*		
4	31.2	CH ₂			
5	30.7	CH ₂			
6	63.9	CH ₂	*		
7	31	CH ₂			
8	23.7	CH ₂			
9	22.4	CH ₂			
10	51.9	CH ₂			
11	125.6	CH	5.45 dd	9 Hz	Trans
12	137.2	CH	6.32 dd	9 Hz	Trans
13	129.5	CH	6.11 dd	10 Hz	Trans
14	137.6	CH	5.8 m		Trans
15	32.6	CH ₂			
16	32	CH ₂			
17	129.9	CH	5.38	1.8 Hz	Cis
18	130.9	CH	5.28	1.8 Hz	Cis
19	34.5	CH ₂			
20	22.6	CH ₂			
21	13.9	CH ₃			

* NOESY demonstrated enhancements for H2 & H6 defining Cis relative stereo chemistry

The Euglenoid toxin as described herein can be produced by: (a) culturing a *Euglena sanguinea* in a growth media to produce Euglenoid toxin therein; (b) separating a first fraction of organic compounds including said Euglenoid toxin from said growth media; (c) separating a second fraction consisting essentially of said Euglenoid toxin from said first fraction by chromatography with porous silica beads.

Growth Studies of Clonal Euglenoid Cultures

Clonal isolates of three *Euglena sanguinea* strains and *Euglena viridis*, *Euglena granulata*, and *Euglena splendens* were grown in AF6 media at 28° C. on a 14:10 L:D cycle. Culture were sampled every 3-5 days for growth rates estimation, and a single mid-exponential phase end point was used to determine toxicity of the other species.

Clonal isolates of five representative cyanoprokaryote, diatom, and green algae were grown in BG11 media at 28 C on a 14:10 L:D cycle. At mid-exponential phase growth, 1 mL aliquots of each culture was transferred to 96-well plates.

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Carrier solvent (control) and euglenophycin toxin was added to three of more replicate vials (at 0, 300 ppb, 3 ppm, and 30 ppm) and growth was monitored for five days.

The following examples are intended to further illustrate the invention, without any intent for the invention to be limited to the specific embodiments described therein. All patents and publications cited herein are incorporated by reference.

Example 1

Cytotoxicity Against Mammalian Tissue Cell Cultures

GH₄C₁ rat pituitary cells as prepared supra, were exposed to euglenophycin. Specifically, *Euglena sanguinea* cells were lyophilized to be dried and then extracted with 200 ml of dichloromethane. The solution was sonicated followed by decanting all solvent leaving the resulting cell mass. The cell mass was transferred with 100 g latrabeads along with 200 ml dichloromethane and rotoevaporated to dryness. The dry extract was then eluted through a column of clean latrabeads with a plurality of solvents as indicated in Table 2. Of the seven fractions, three fractions displayed activity against GH₄C₁ rat pituitary cells.

TABLE 2

Solvent	GH ₄ C ₁ rat pituitary cells activity
100% Toluene	No
50%-50% Toluene-Ethyl Acetate	Yes
100% Ethyl Acetate	Yes
50%-50% Ethyl Acetate-Acetone	Yes
100% Acetone	No
50%-50% Acetone-Methanol	No
100% Methanol	No

Example 2

Allelopathy Against Tissue Cell Cultures

To evaluate the euglenophycin against the growth rate of selected algal taxa. Cultures of five algal species grown in batch culture were exposed to the euglenophycin. The culture include *Microcystis aeruginosa* (cyanobacteria), *Planktothrix* (cyanobacteria), *Gomphonema parvum* (diatom), *Scenedesmus dimorphus* (green algae), and *Oocystis polymorpha* (green algae). The cultures were grown using 14:10 hour L:D cycles, in BG11 culture media. When the alga reached exponential phase, the cells were dispensed into Falcon 96-well tissue culture plates and were dosed with euglenophycin at 0, 0.3, 3 and 30 mg/L concentrations. Solvent blanks (acetone) were included in the control test. Readings of chlorophyll a were made on a BMG Labtech FLUOstar Omega spectrometer daily for four days. FIG. 2 depicts that all cultures were negatively affected by euglenophycin exposure.

Example 3

Toxicity Against Adenocarcinoma Cell Line, Ht-29

ATCC culture collection HTB-38, also termed Ht-29, was tested against the *Euglena sanguinea* derived toxin as indicated in FIG. 3. Specifically, cancer cells were grown to mid-exponential growth phase, aliquots were added to tissue